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(71) Applicant: WHITEHEAD INSTITUTE FOR BIOMEDICAL RESEARCH [US/US]; Nine Cambridge Center, Cambridge, MA 02142 (US).

(72) Inventors: LIN, Herbert, Y. ; 550 Memorial Drive, Apt. 12D1, Cambridge, MA 02139 (US). WANG, Xiao-Fan ; 872 Massachusetts Avenue, № 401, Cambridge, MA 02139 (US). WEINBERG, Robert, A. ; 25 Copley Street, Brookline, MA 02146 (US). LODISH, Harvey, F. ; 195 Fisher Avenue, Brookline, MA 02146 (US).

(74) Agents: GRANAHAN, Patricia et al.; Hamilton, Brook, Smith & Reynolds, Two Militia Drive, Lexington, MA 02173 (US).

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(54) Title: TGF-β TYPE RECEPTOR cDNAS AND USES THEREFOR

(57) Abstract

DNA encoding TGF-β type III receptor of mammalian origin, DNA encoding TGF-β type II receptor of mammalian origin, TGF-β type III receptor, TGF-β type II receptor and uses therefor.

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TGF-β TYPE RECEPTOR cDNAs AND USES THEREFOR

Description

Background

Transforming growth factor-beta (TGF-β) is a member of a family of structurally related cytokines that elicit a variety of responses, including growth, differentiation, and morphogenesis, in many different cell types. (Roberts, A.B. and M.B. Sporn, In: Peptide Growth Factors and Their Receptors, Springer-Verlag, Heidelberg, pp. 421-472 (1990); Massague, J., Annu. Rev. Cell. Biol. 6:597-641 (1990)) In vertebrates at least five different forms of TGF-β, termed TGF-β1 to TGF-β5, have been identified; they all share a high degree (60%-80%) of amino-acid sequence identity. While TGF-β1 was initially characterized by its ability to induce anchorage-independent growth of normal rat kidney cells, its effects on most cell types are anti-mitogenic. (Altschul, S.F. et al., J. Mol. Biol. 215:403-410 (1990); Andres, J.L. et al., J. Cell. Biol. 109:3137-3145 (1989)). It is strongly growth-inhibitory for many types of cells, including both normal and transformed epithelial, endothelial, fibroblast, neuronal, lymphoid, and hematopoietic cells. In addition, TGF-β plays a central role in regulating the formation of extracellular matrix and cell-matrix adhesion processes.

In spite of its widespread effects on cell phenotype and physiology, little is known about the biochemical mechanisms that enable TGF-β family members to elicit these varied responses. Three distinct high-affinity

cell-surface TGF-β-binding proteins, termed type I, II and III, have been identified by incubating cells with radiolabelled TGF-β1, cross-linking bound TGF-β1 to cell surface molecules, and analyzing the labelled complexes by polyacrylamide gel electrophoresis. (Massague, J. and B. Like, J. Biol. Chem. 260:2636-2645 (1985); Cheifetz, S. et al. J. Biol. Chem. 261:9972-9978 (1986).) The binding constants are about 5-50pM for the type I and II receptor and 30-300 pM for the type III receptor. (Boyd, F.T. and J. Massague, J. Biol. Chem. 264:2272-2278 (1989))

The type I and II receptors, of estimated 53 and 70-100 kilodaltons mass respectively, are N-glycosylated transmembrane proteins that are similar in many respects. Each of these receptors has a distinct affinity for each member of the TGF-β family of ligands. (Boyd, F.T. and J. Massague, J. Biol. Chem. 264:2272-2278 (1989)) In contrast, the type III receptor shows comparable affinities for all TGF-β isotypes; the type III receptor is the most abundant cell-surface receptor for TGF-β in many cell lines (upwards of 200,000 per cell), and is an integral membrane proteoglycan. It is heavily modified by glycosaminoglycan (GAG) groups, and migrates heterogeneously upon gel electrophoresis as proteins of 280 to 330 kilodaltons. When deglycosylated with heparitinase and chondrontinase, the protein core migrates as a 100-110 kilodalton protein. The TGF-β binding site resides in this protein core, as non-glycosylated forms of this receptor that are produced in cell mutants defective in GAG synthesis are capable of ligand binding with affinities comparable to those of the natural receptor. (Cheifetz, S. and J. Massague, J. Biol. Chem., 264:12025-12028 (1989) A variant form of type III

receptor is secreted by some types of cells as a soluble molecule that apparently lacks a membrane anchor. This soluble species is found in low amounts in serum and in extracellular matrix.

The type III receptor, also called betaglycan, has a biological function distinct from that of the type I and II receptors. Some mutant mink lung epithelial cell (Mv1Lu) selected for loss of TGF-β responsiveness no longer express type I receptors; others, similarly selected, lose expression of both the type I and II receptors. However, all these variants continue to express the type III receptor. (Boyd, F.T. and J. Massague, J. Biol. Chem. 264:2272-2278 (1989); Laiho, M. et al., J. Biol. Chem. 265:18518-18524 (1990)) This has led to the proposal that types I and II receptors are signal-transducing molecules while the type III receptor, may subserve some other function, such as in concentrating ligand before presentation to the bona fide signal-transducing receptors. The secreted form of type III receptor, on the other hand, may act as a reservoir or clearance system for bioactive TGF-β.

Additional information about each of these TGF-β receptor types would enhance our understanding of their roles and make it possible, if desired, to alter their functions.

Summary of the Invention

The present invention relates to isolation, sequencing and characterization of DNA encoding the TGF-β type III receptor of mammalian origin and DNA encoding the TGF-β type II receptor of mammalian origin. It also relates to the encoded TGF-β type III and type II receptors, as well as to the soluble form of each; uses

of the receptor-encoding genes and of the receptors themselves; antibodies specific for TGF-β type III receptor and antibodies specific for TGF-β type II receptor. In particular, it relates to DNA encoding the TGF-β type III receptor of rat and human origin, DNA encoding the TGF-β type II receptor of human origin and homologues of each.

The TGF-β receptor-encoding DNA of the present invention can be used to identify equivalent TGF-β receptor type III and type II genes from other sources, using, for example, known hybridization-based methods or the polymerase chain reaction. The type III receptor gene, the type II receptor gene or their respective encoded products can be used to alter the effects of TGF-β (e.g., by altering receptivity of cells to TGF-β or interfering with binding of TGF-β to its receptor), such as its effects on cell proliferation or growth, cell adhesion and cell phenotype. For example, the TGF-β receptor type III gene, the TGF-β receptor type II gene, or a truncated gene which encodes less than the entire receptor (e.g., soluble TGF-β type III receptor, soluble TGF-β type II receptor or the TGF-β type III or type II binding site) can be administered to an individual in whom TGF-β effects are to be altered. Alternatively, the TGF-β type III receptor, the TGF-β type II receptor, a soluble form thereof (i.e., a form lacking the membrane anchor) or an active binding site of the TGF-β type III or the type II receptor can be administered to an individual to alter the effects of TGF-β.

Because of the many roles TGF-β has in the body, availability of the TGF-β receptors described herein makes it possible to further assess TGF-β function utilizing <u>in vivo</u> as well as <u>in vitro</u> methods and to alter (enhance or diminish) its effects.

Brief Description of the Drawings

Figure 1 is the DNA sequence (SEQ ID NO. 1) and the translated amino acid sequence (SEQ ID NO. 2) of type III TGF-β1 receptor cDNA clone R3-OFF (full insert size 6 kb), in which the open reading frame with flanking sequences of the clone are shown. The transmembrane domain is indicated by a single underline. Peptide sequences from purified type III receptor, mentioned in text, that correspond to the derived sequence, are in italics and underlined. Potential N-linked glycosylation sites are indicated by #, and extracellular cysteines by &. A consensus protein kinase C phosphorylation site is indicated by $. The last non-vector encoded amino acid of Clone R3-OF (2.9 kb) is indicated by @. Consensus proteoglycan attachment site is indicated by +++. Other potential glycosaminoglycan attachment sites are indicated by +. The upstream in-frame stop codon (-42 to -44) is indicated by a wavy line. Signal peptide cleavage site predicted by vonHeijne's algorithm (von Heijne, G., Nucl. Acid. Res. 14:4683-4690 (1986) is indicated by an arrow.

Figure 2 is the nucleotide sequence of the full-length type II TGF-β receptor cDNA clone 3FF isolated from a human HepG2 cell cDNA library (full insert size 5 kb) (SEQ ID NO. 3). The cDNA has an open reading frame encoding a 572 amino acid residue protein.

Figure 3 is the amino acid sequence of the full-length type II TGF-β receptor (SEQ ID NO. 4).

Detailed Description of the Invention

The subject invention is based on the isolation and sequencing of DNA of vertebrate, particularly mammalian, origin which encodes TGF-β type III receptor and DNA of mammalian origin which encodes TGF-β type II receptor,

expression of the encoded products and characterization of the expressed products. As described, a full-length cDNA which encodes TGF-β receptor type III has been isolated from a cDNA library constructed from a rat vascular smooth muscle cell line and a full-length cDNA which encodes TGF-β type II receptor has been isolated from a human cDNA library. The human homologue of the type III gene has also been cloned. A deposit of human TGF-β type III cDNA in the plasmid pBSK has been made under the terms of the Budapest Treaty at the American Type Culture Collection (10/21/91) under Accession Number 75127. All restrictions upon the availability of the deposited material will be irrevocably removed upon granting of a U.S. patent based on the subject application.

<u>Isolation and Characterization of TGF-β Type III Receptor</u>

As described herein, two separate strategies were pursued for the isolation of the TGF-β type III receptor cDNA. In one approach, monoclonal antibodies were generated against the type III receptor protein and used to purify the receptor, which was then subjected to microsequencing. (See Example 1) Microsequencing of several peptides resulting from partial proteolysis of the purified receptor produced four oligopeptide sequences, which were used to construct degenerate oligonucleotides. The degenerate oligonucleotides were used either as primers in a cloning strategy using the polymerase chain reaction (PCR) or as probes in screening cDNA libraries. Although this strategy did not prove to be productive, the oligopeptide sequences were useful in verifying the identity of the receptor clones isolated by the second strategy.

In the second approach to isolating TGF-β receptor-encoding clones, an expression cloning strategy was used in COS cells; direct visualization of receptor positive cells was used to isolate receptor cDNAs. (See Example 2) In this approach, a cDNA library was constructed from A-10 cells, a rat vascular smooth muscle cell line which expresses all three TGF-β receptors (type I, II and III). COS cells transfected with cDNA components of this library in a vector carrying the cytomegalovirus (CMV) transcriptional promoter and the SV40 origin of replication were screened to identify cells expressing substantially higher than normal levels of TGF-β receptor. One transfectant expressing such high levels of a TGF-β binding protein was identified and the original pool of expression constructs from which it was derived was split into subpools, which were subjected to a second round of screening. Two further rounds of sib-selection resulted in isolation of one cDNA clone (R3-OF) with a 2.9 kb insert which induced high levels of TGF-β-binding proteins in approximately 10% of cells into which it was introduced. The specificity of the TGF-β binding was validated by showing that addition of a 200-fold excess unlabeled competitor TGF-β1 strongly reduced binding of 125 I-TGF-β to transfected cells.

The R3-OF cDNA encoded an open reading frame of 817 amino acid residues, but did not contain a stop codon. R3-OF was used as a probe to isolate a full-length cDNA from a rat 208F library. The resulting clone, R3-OFF, is 6kb in length and encodes a protein of 853 amino acids, which is colinear with clone R3-OF. The nucleotide sequence of R3-OFF is shown in Figure 1, along with the translated amino acid sequence.

Characterization of the receptor encoded by R3-OFF was carried out, as described in Example 3. Results showed three distinct TGF-β binding protein species of TGF-β on the surface of mock-transfected COS cells, which is in accord with results reported by others. (Massague, J. et al., Ann. NY Acad. Sci. 593:59-72 (1990)). These included the two lower molecular weight type I and II receptors (65 and 85 kD) and the higher molecular weight type III proteoglycan, which migrates as a diffuse band of 280-330 kd. Enzymatic removal of the proteoglycan yielded a core protein of approximately 100 kd. Binding to all three receptor types is specific in that it was competed by 200-fold excess of unlabeled TGF-β1.

Transfecting the isolated cDNA caused a two-fold increase in expression of the type III receptor. When a cell lysate derived from COS cells transfected with clone R3-OFF was treated with deglycosylating enzymes, the heterogeneous 280-330 kd band was converted to a protein core which co-migrates with the type III protein core seen in parental A10 cells. Importantly, the recombinant protein core migrated differently from the endogenous COS cell type III protein core.

These observations were confirmed and extended using stably transfected cells expressing the type III cDNA. L6 rat skeleton muscle myoblasts do not express any detectable type III mRNA and no endogeneous surface type III receptor (Massague et al., 1986; Segarini et al., 1989). These cells were transfected with the isolated cDNA in the vector pcDNA-neo. Cell clones stably expressing this clone in both the forward and reverse orientations with respect to the CMV promoter were isolated and analyzed by ligand binding assay.

Introduction of either the full-length clone R3-OFF or the partial clone R3-OF in the forward orientation resulted in expression of type III receptor. L6 cells transfected with the cDNA clones in the reverse orientation did not express this protein. Importantly, the apparent size of the protein core of the type III receptor in cells transformed with the R3-OF clone is smaller than that from R3-OFF transformed cells, consistent with the difference in the sizes of the protein cores predicted from their nucleic acid sequences.

Surprisingly, binding of radio-labeled ligand to the type II receptor was increased by 2.5 fold in cells expressing the type III cDNA. Binding to the type I receptor was unchanged. This apparently specific up-regulation of ligand-binding to the type II receptor was evident in all of the 15 stably transfected L6 cell lines analyzed to date. Furthermore, this effect seems to be mediated equally well by the full-length clone or a truncated clone (R3-OF) that lacks the cytoplasmic domain of TGF-β type III receptor was expressed.

Expression of type III receptor mRNA was assessed by Northern blot analysis and RNA blot analysis. Northern gel analysis showed that the type III receptor mRNA is expressed as a single 6 kb message in several rat tissues. RNA dot blot analysis of several different tissue culture cell lines was also carried out. Cells of mouse origin (MEL and YH16) appear to express a smaller (~5.5 kb) message for the type III mRNA than those of pig, rat and human origin. In all of these cells, expression or absence of the type III mRNA is consistent with the expression or absence of detectable cell surface

type III receptors, with the notable exception of the retinoblastoma cell lines (Y79, Weri-1, Weri-24, and Weri-27). These cells lack detectable surface expression of type III receptor, which confirms an earlier report. (Kimchi, A. et al., Science 240:196-198 (1988)). It is striking that the type III receptor mRNA is expressed in these cells at a level comparable to that of other cells that do indeed express type III receptor proteins at readily detectable levels. It appears that TGF-β receptor III expression, which is substantial in normal retinoblasts (AD12), has been down-regulated in these retinoblastoma tumor cells, perhaps through post-transcriptional mechanisms.

The nucleotide sequence full reading frame along with flanking sequences of the full-length cDNA clone R3-OFF was determined and is presented in Figure 1. The reading frame encodes a protein of 853 amino acid residues, which is compatible with the 100 kD size observed for the fully deglycosylated TGF-β1 type III receptor. The identity of the receptor as TGF-β type III was verified by searching for segments of the putative transcription product which included the peptide sequences determined by microsequencing of the isolated type III receptor. (See Example 1) As indicated in Figure 1, two segments of derived protein (underlined and italicized, residues 378-388 and 427-434) precisely match with the amino acid sequences of two peptides (I and III) determined from direct biochemical analysis of the purified type III receptor.

Further analysis showed that TGF-β type III binding protein has an unusual structure for a cytokine receptor. Hydropathy analysis indicates that the protein includes a

N-terminal signal sequence, followed by a long, hydrophilic N-terminal region. A 27 residue region of strong hydrophobicity (underlined in Figure 1, residues 786-812) toward the C-terminus represents the single putative transmembrane domain. This suggests that nearly all of the receptor which is an N-terminal extracellular domain is anchored to the plasma membrane near its C-terminus. A relatively small C-terminal tail of 41 residues represents the cytoplasmic domain.

Analysis of related sequences provides few clues to function of TGF-β type III protein. Only one other gene described to date, a glycoprotein expressed in high quantities by endothelial cells and termed endoglin, contains a related amino acid sequence. The most homologous regions between the sequences of the type III receptor and endoglin (74%) falls primarily in the putative transmembrane and cytoplasmic domains. Similar to the general structure of type III receptor, endoglin is a glycoprotein which contains a large hydrophilic N-terminal domain which is presumably extracellular, followed by a putative transmembrane domain and a short cytoplasmic tail of 47 amino acid residues. The biological role of endoglin is still unclear at present, although it has been suggested that it may involved in cell-cell recognition through interactions of an "RGD" sequence on its ectodomain with other adhesion molecules. Unlike the TGF-β type III receptor, endoglin does not carry GAG groups.

Isolation of TGF-β Type II Receptor

The cDNA encoding the type II TGF-β receptor was also isolated, using expression cloning in COS cells. A full-length cDNA (designated clone 3FF) was isolated by high stringency hybridization from a human HepG2 cell cDNA library. (See Example 6) Analysis showed that the corresponding message is a 5 kb message which is expressed in different cell lines and tissues. Sequence analysis indicated that the cDNA has an open reading frame encoding a core 572 amino acid residue protein. The nucleotide sequence of the full-length type II TGF-β receptor cDNA clone 3FF is shown in Figure 2; the amino acid sequence is represented in Figure 3.

The 572 amino acid residue protein has a single putative transmembrane domain, several consensus glycosylation sites, and a putative intracellular serine/threonine kinase domain. The predicted size of the encoded protein core is ~60 kd, which is too large for a type I TGF-β receptor. Instead, crosslinking experiments using iodinated TGF-β and COS cells transiently transfected with clone 3FF shows over-expression of a protein approximately 70-80 kd which corresponds to the size of type II TGF-β receptors. Thus, clone 3FF encodes a protein that specifically binds TGF-β and has an expressed protein size of 70-80 kd, both characteristic of the type II TGF-β receptor.

Uses of the Cloned TGF-β Receptors and Related Products

For the first time, as a result of the work described herein, DNAs encoding two of the three high affinity cell-surface TGF-β receptors have been isolated, their sequences and expression patterns determined and

the encoded proteins characterized. Expression of the TGF-β type III receptor in cells which do not normally express the receptor, followed by ligand binding assay, verified that the cloned type III receptor-encoding DNA (i.e., either the full-length clone R3-OFF or the partial clone R3-OF) encoded the receptor. In addition, the work described herein resulted in the surprising finding that binding of TGF-β to type II receptors in cells expressing the type III DNA was increased by 2.5 fold.

Additional insight into the role of the TGF-β type III receptor and its interaction with TGF-β type II receptor is a result of the work described. For example, the role of TGF-β type III receptor is unclear, but it has been proposed that it serves a most unusual function of attracting and concentrating TGF-βs for eventual transfer to closely situated signal-transducing receptors. While most cytokines bind to a single cell surface receptor, members of the TGF-β family bind with greater or lesser affinity to three distinct cell surface proteins. This has raised the question of why these three receptors are displayed by most cell types and whether they subserve distinct functions. Evidence obtained to date suggests that the type III receptor may perform functions quite different from those of types I and II. Thus, type III is substantially modified by GAGs while types I and II appear to carry primarily the N-linked (and perhaps O-linked) sidechains that are characteristic of most growth factor receptors. In addition, variant cells that have been selected for their ability to resist TGF-β-induced growth inhibition show the absence of Type I or Type II receptors while continuing to display Type III receptors. Together, these data have caused some to propose that the Type I

and II receptors represent bona fide signal-transducing receptors while the type III receptor, described here, plays another distinct role in the cell.

It remains possible that the type III receptor serves a most unusual function of attracting and concentrating TGF-βs on the cell surface for eventual transfer to closely situated signal-transducing receptors. Such a function would be unprecedented for a proteinaceous receptor, although heparin sulfate has been shown to activate basic FGF by binding to this growth factor prior to FGF association with its receptor (Yayon, A. et al., Cell 64:841-848 (1991)) Parenthetically, since the type III receptor also contains large quantities of heparan sulfate side-chains, it may also bind and present basic FGF to its receptor.

Evidence that is consistent with the role for the type III receptor comes from the work with L6 rat myoblast cells which is described herein. As described above, in L6 cells overexpressing type III receptor, the binding of radiolabelled TGF-β to the type II receptor is increased several fold when compared with that seen with parental cells. Further assessment of TGF-β type III function and interaction with type II and type I receptors will be needed to answer these questions and can be carried out using the materials and methods described here.

TGF-β receptors, both type III and type II, can be identified in other species, using all or a portion of the DNA encoding the receptor to be identified as a probe and methods described herein. For example, all or a portion of the DNA sequence encoding TGF-β type III receptor (shown in Figure 1) or all or a portion of the

DNA sequence encoding TGF-β type II receptor (shown in Figure 2) can be used to identify equivalent sequences in other animals. Stringency conditions used can be varied, as needed, to identify equivalent sequences in other species. Once a putative TGF-β receptor type III or type II-encoding sequence has been identified, whether it encodes the respective receptor type can be determined using known methods, such as described herein for verification that the cDNA insert of full-length clone R3-OFF and the cDNA insert of partial clone R3-OF encode the type III receptor. For example, DNA isolated in this manner can be expressed in an appropriate host cell which does not express the receptor mRNA or the surface receptor (e.g., L6 rat skeleton muscle myoblasts) and analyzed by ligand binding (TGF-β binding) assay, as described herein.

Also as a result of the work described herein, antibodies (polyclonal or monoclonal) specific for the cloned TGF-β type III or the clones TGF-β type II receptor can be produced, using known methods. Such antibodies and host cells (e.g., hybridoma cells) producing the antibodies are also the subject of the present invention. Antibodies specific for the cloned TGF-β receptor can be used to identify host cells expressing isolated DNA thought to encode a TGF-β receptor. In addition, antibodies can be used to block or inhibit TGF-β activity. For example, antibodies specific for the cloned TGF-β type III receptor can be used to block binding of TGF-β to the receptor. They can be administered to an individual for whom reduction of TGF-β binding is desirable, such as in some fibrotic diseases (e.g., of skin, kidney and lung).

The method of the present invention can be used for diagnosis of disorders involving abnormal binding of TGF-β to TGF-β type III receptors and/or TGF-β type II receptors, such as fibrotic diseases. Abnormal binding of TGF-β to TGF-β type III receptor or TGF-β type II receptor at a cell surface may be measured, resulting in a test binding value, which is compared to an appropriate control binding value. Control binding values can be obtained using control cells known to have abnormal binding of TGF-β to its receptors or control cells which are normal cells (e.g., evidence TGF-β binding to the TGF-β receptor is within physiological levels). Control values are obtained by determining the extent to which TGF-β binds the appropriate receptor (i.e., TGF-β type III receptor or TGF-β type II receptor); such values can be obtained at the time the test binding value is determined or can be previously determined (i.e., a previously determined standard). A test binding value similar to the control binding value obtained from abnormal cells is indicative of abnormal binding of TGF-β to TGF-β type III receptor or TGF-β type II receptor. A test binding value similar to the control binding value obtained from normal cells is indicative of normal binding of TGF-β to TGF-β type III receptor or TGF-β type II receptor.

DNA and RNA encoding TGF-β type III receptor and DNA and RNA encoding TGF-β type II receptor are now available. As used herein, the term DNA or RNA encoding the respective TGF-β receptor includes any oligodeoxynucleotide or oligodeoxyribonucleotide sequence which, upon expression, results in production of a TGF-β receptor having the functional characteristics of the

TGF-β receptor. That is, the present invention includes DNA and RNA which, upon expression in an appropriate host cell, produces a TGF-β type III receptor which has an affinity for TGF-β similar to that of the TGF-β type III receptor on naturally occurring cell surfaces (e.g., it shows comparable affinities for all TGF-β isotypes). Similarly, the present invention includes DNA and RNA which, upon expression in an appropriate host cell, produces a TGF-β type II receptor which has an affinity for TGF-β similar to that of TGF-β type II receptor on naturally occurring cell surfaces (e.g., it has a distinctive affinity for each member of the TGF-β family of ligands similar to that of the naturally occurring TGF-β type II receptor). The DNA or RNA can be produced in an appropriate host cell or can be produced synthetically (e.g., by an amplification technique such as PCR) or chemically.

The present invention also includes the isolated TGF-β type III receptor encoded by the nucleotide sequence of full-length R3-OFF, the isolated TGF-β type III receptor encoded by the nucleotide sequence of partial clone R3-OF, the isolated TGF-β type II receptor encoded by the nucleotide sequence of full-length clone 3FF and TGF-β type III and type II receptors which bind TGF-β isotypes with substantially the same affinity. The isolated TGF-β type III and type II receptors can be produced by recombinant techniques, as described herein, or can be isolated from sources in which they occur naturally or synthesized chemically. As used herein, the terms cloned TGF-β type III and cloned TGF-β type II receptors include the respective receptors identified as

described herein, and TGF-β type III and type II receptors (e.g., from other species) which exhibit substantially the same affinity for the TGF-β isotypes as the respective receptors.

As described previously, cells in which the cloned TGF-β type III receptor is expressed bind TGF-β in essentially the same manner as do cells on which the type III receptor occurs naturally. Further analysis of ligand interactions with the cloned TGF-β type III receptor, based upon site-directed mutagenesis of both TGF-β and the receptor, can be carried out to identify residues important for binding. For example, DNA having the sequence of Figure 1 can be altered by adding, deleting or substituting at least one nucleotide, in order to produce a modified DNA sequence which encodes a modified cloned TGF-β type III receptor. The functional characteristics of the modified receptor (e.g., its TGF-β-binding ability and association of the binding with effects normally resulting from binding) can be assessed, using the methods described herein. Modification of the cloned TGF-β type III receptor can be carried out to produce, for example, a form of the TGF-β type III receptor, referred to herein as soluble TGF-β receptor, which is not membrane bound and retains the ability to bind the TGF-β isotypes with an affinity substantially the same as the naturally-occurring receptor. Such a TGF-β type III receptor could be produced, using known genetic engineering or synthetic techniques; it could include none of the transmembrane region present in the naturally-occurring TGF-β type III receptor or only a small portion of that region (i.e., small enough not to

interfere with its soluble nature). For example, it can include amino acids 1 through 785 of the TGF-β type III sequence of Figure 1 or a portion of that sequence sufficient to retain TGF-β binding ability (e.g., amino acids 24-785, which does not include the signal peptide cleavage site present in the first 23 amino acids). A soluble TGF-β type II receptor (e.g., one which does not include the transmembrane and cytoplasmic domains) can also be produced. For example, it can include amino acids 1 through 166, inclusive, of Figure 3 or a sufficient portion thereof to retain TGF-β binding ability substantially the same as that of TGF-β type II receptor.

The TGF-β type III receptor and/or type II receptor can be used for therapeutic purposes. As described above, the TGF-β family of proteins mediate a wide variety of cellular activities, including regulation of cell growth, regulation of cell differentiation and control of cell metabolism. TGF-β may be essential to cell function and most cells synthesize TGF-β and have TGF-β cell surface receptors. Depending on cell type and environment, the effects of TGF-β vary: proliferation can be stimulated or inhibited, differentiation can be induced or interrupted and cell functions can be stimulated or suppressed. TGF-β is present from embryonic stages through adult life and, thus, can affect these key processes throughout life. The similarities of a particular TGF-β (e.g., TGF-β1) across species and from cell to cell are considerable. For example, the amino acid sequence of a particular TGF-β and the nucleotide sequence of the gene which encodes it regardless of

source, are essentially identical across species. This further suggests that TGF-β has a critical role in essential processes.

Specifically, TGF-β has been shown to have anti-inflammatory and immune suppression capabilities, to play an important role in bone formation (by increasing osteoblast activity), inhibit cancer cell proliferation in culture, and control proliferation of glandular cells of the prostate. As a result, it has potential therapeutic applications in altering certain immune system responses (and possibly in modifying immune-mediated diseases); in treating systemic bone disease (e.g., osteoporosis) and conditions in which bone growth is to be enhanced (e.g., repair of broken bones) and in controlling growth and metastasis of cancer cells. In addition, TGF-β appears to play a role in determining whether some cell types undergo or do not undergo mitosis. In this respect, TGF-β may play an important role in tissue repair. Some diseases or conditions appear to involve low production or chronic overproduction of TGF-β. (For example, results of animal studies suggest that there is a correlation between the over production of TGF-β and diseases characterized by fibrosis in the lung, kidney, liver or in viral mediated immune expression.)

Clearly, TGF-β has key roles in body processes and numerous related potential clinical or therapeutic applications in wound healing, cancer, immune therapy and bone therapy. Availability of TGF-β receptor genes, the encoded products and methods of using them *in vitro* and *in vivo* provides an additional ability to control or

regulate TGF-β activity and effect in the body. For example, the TGF-β type II or type III receptor encoded by the type II or the type III receptor genes of the subject invention can be used, as appropriate, to alter the effects of TGF-β (e.g., to enhance the effect of TGF-β in the body or to inhibit or reduce (totally or partially) its effects). It is also possible to administer to an individual in whom TGF-β bound to TGF-β type III receptor, such as soluble TGF-β type III receptor. The present invention provides both a TGF-β agonist and a TGF-β antagonist. For these purposes, DNA gene encoding the entire TGF-β type II or type III receptor, the encoded type II or type III receptor or a soluble form of either receptor can be used. Alternatively, antibodies or other ligands designed based upon these sequences or specific for them can be used for this purpose.

Knowledge of the amino acid sequences of TGF-β type III and type II receptors makes it possible to better understand their structure and to design compounds which interfere with binding of the receptor with TGF-β. It makes possible identification of existing compounds and design of new compounds which are type III and/or type II receptor antagonists.

Cells expressing the type III and/or type II receptors of the present invention can be used to screen compounds for their ability to interfere with (block totally or partially) TGF binding to the receptors. For example, cells which do not express TGF-β type III receptor (e.g., L6 rat skeleton muscle myoblasts) but have been modified to do so by incorporation of the type III cDNA in an appropriate vector can be used for this

purpose. A compound to be assessed is added, for example, to tissue culture dishes containing type III expressing cells, along with labeled TGF-β. As a control, the same concentration of labeled TGF-β is added to tissue culture dishes containing the same type of cells. After sufficient time for binding of TGF-β to the receptor to occur, binding of labeled TGF-β to the cells is assessed, using known methods (e.g., by means of a gamma counter) and the extent to whcih it occurred in the presence and in the absence of the compound to be assessed is determined. Comparison of the two values show whether the test compound blocked TGF-β binding to the receptor (i.e., less binding in the presence of the compound than in its absence is evidence that the test compound has blocked binding of TGF-β to the TGF-β type III receptor).

Alternatively, a cell line expressing the TGF-β receptor or cells expressing microinjected TGF-β receptor RNA, can be used to assess compounds for their ability to block TGF-β binding to the receptor. In this embodiment, a compound to be assessed is added to tissue culture dishes containing the cell line cells expressing microinjected TGF-β receptor RNA, along with TGF-β. As a control, TGF-β alone is added to the same type of cells expressing microinjected endothelin receptor RNA. After sufficient time for binding of TGF-β to the receptor to occur, the extent to which binding occurred is measured, both in the presence and in the absence of the compound to be assessed. Comparison of the two values shows whether the compound blocked TGF-β binding to the receptor. The TGF-β type III and type II receptors can

also be used to identify TGF-β-like substances, to purify TGF-β and to identify TGF-β regions which are responsible for binding to the respective receptors. For example, the type III receptor can be used in an affinity-based method to identify substances which bind the receptor in a manner similar to TGF-β.

The invention will now be illustrated by the following examples, which are not intended to be limiting in any way.

EXAMPLES

Materials and methods used in Examples 1-5 are described below.

Materials

The following is a description of materials used in the work described herein.

Recombinant human TGF-β1 was provided by Rik Derynck of Genentech. COS-M6 cells were provided by Brian Seed of the Massachusetts General Hospital and Alejandro Aruffo of Bristol-Myers-Squibb. Heparitinase was provided by Tetsuhito Kojima and Robert Rosenberg of MIT. LLC-PK_1 cells were a gift of Dennis Ausiello of the Massachusetts General Hospital. YH-16 cell were a gift of Edward Yeh of the Massachusetts General Hospital. 3-4 cells were a gift of Eugene Kaji of the Whitehead Institute for Biomedical Research. All other cell lines were purchased from ATCC and grown as specified by the supplier, except as noted.

Expression Cloning

Construction of cDNA Library and Generation of Plasmid Pools

10μg polyadenylated mRNA was prepared from A10 cells by the proteinase-K/SDS method (Gonda et al., Molec. Cell. Biol. 2:617-624 (1982)). Double stranded cDNA was synthesized and linkered to nonpalindromic BstX1 adaptors as described by Seed, B. and A. Aruffo, Proc. Natl. Acad. Sci. USA 84:3365-3369 (1987). Acaptored cDNA was size-fractionated on a 5 to 20% potassium acetate gradient, and inserts greater than 1 kb were ligated to the plasmid vector pcDNA-1, and electroporated in the E. coli MC1061/P3, yielding a primary library with a titer of $>10^7$ recombinants. A portion of the cDNA was plated as pools of $\sim 1\times 10^4$ recombinant bacteria colonies grown on 15 cm petri dishes with Luria-broth agar containing 7.5 mg/ml tetracycline and 12.5 mg/ml ampicillin. Cells were scraped off the plates in 10 mls of Luria-broth, and glycerol stocks of pooled bacteria were stored at -70°C. The remaining bacteria was used to purify plasmid DNA using the alkaline lysis mini-prep method (Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, 2d Ed. (Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press (1989)).

COS Cell Transfections and Binding Assay

Plasmid pools (each representing $\sim 1\times 10^4$ clones) were transfected into COS-M6 (subclone of COS-7 cells) by the DEAE-dextran/chloroquine method described by Seed, B. and A. Aruffo, Proc. Natl. Acad. Sci. USA 84:3365-3369 (1987). Forty-eight hours after transfection, cells were

incubated with 50 pM^{125}I-TGF-β1 (100 to 200 Ci/mmol) for 4 hours at 4°C), autoradiographic analysis of transfected cells was performed using NT-B2 photographic emulsion (Kodak) essentially as described (Gearing, D.P. et al., EMBO J. 8:3667-3676 (1989)). After development of slides, cells were air-dried and mounted with mounting media and a glass coverslip. Slides were analyzed under an Olympus OM-T1 inverted phase-contrast microscope using a dissection trans-illuminator for darkfield illumination.

Subdivision of Positive Pool

Of 86 pools screened, one pool (#13) was identified as positive and a glycerol stock of bacteria corresponding to this pool was titered and 25 pools of 1000 clones were generated and miniprep plasmid from these pools were transfected into COS cells as described above. Several positive pools of 1000 were identified, and one was replated as 25 plates of 100 colonies. A replica was made of this positive plate and harvested. Once a positive pool was identified, individual colonies were picked from the corresponding master plate and grown overnight in 3 ml liquid culture. A 2-dimensional grid representing the 100 clones was generated and a single clone, R3-OF, was isolated.

Cloning of R3-OFF

A 208F rat fibroblast library in lambda ZAP II (Stratagene) was screened at high stringency with clone R3-OF insert, and several clones with ~6kb inserts were isolated, one of which is referred to as R3-OFF.

DNA Sequencing and Sequence Analysis

Double-stranded DNA was sequenced by the dideoxy. chain termination method using Sequenase reagents (United States Biochemicals). Comparison of the sequence to the data bases was performed using BLAST (Altschcul, S.F. et al., J. Mol. Biol. 215:403-410 (1990)).

Iodination of TGF-β

TGF-β1 was iodinated using the chloramine-T method as described (Cheifetz, S. and J.L. Andres, J. Biol. Chem. 263:16984-16991 (1988)).

Chemical Cross-Linking

Transfected COS cells grown on 10 cm dishes or subconfluent L6 and A-10 cells grown on 3.5 cm dishes were incubated with ^{125}I-TGF-β1 in binding buffer (Frebs-Ringer buffered with 20 mM Hepes, pH 7.5, 5 mM $MgSO_4$, 0.5% BSA), washed 4 times with ice-cold binding buffer without BSA, and incubated for 15 minutes with binding buffer without BSA containing 60ng/ml disuccinimidyl suberate at 4°C under constant rotation. Crosslinking was terminated by addition of 7% sucrose in binding buffer. Cells were scraped, collected and pelleted by centrifugation, then resuspended in lysis buffer (10 mM Tris, pH 7.4, 1 mM EDTA, pH 8.0, 1% Triton-X 100, 10 μg/ml of pepstatin, 10μg/ml leupeptin, 10 μg/ml antipain, 100 μg/m; benzamidine hydrochloride, 100 μg/ml soybean trypsin inhibitor, 50 μg/ml aprotonin, and 1 mM phenylmethylsulfonyl fluoride). Solubilized material was analyzed by 7% SDS-PAGE and subjected to

autoradiographic analysis by exposure to X-AR film (Kodak) at -70°C.

Enzymatic Digestion

Digestion of solubilized TGF-b receptors with chondroitinase and heparitinase was performed as described (Cheifetz, S. and J.L. Andres, J. Biol. Chem. 263:16984-16991 (1988); Segarini, P.R. and S.M. Seyedin, J. Biol. Chem., 263: 8366-8370 (1988).

Generation of Stable Cell Lines

L6 myoblasts were split 1:10 into 10 cm dishes and transfected the following day by the calcium phosphate method (Chen, C. and H. Okayama, Molec. Cell. Biol. 7:2745-2752 (1987)) with clones R3-OF or R3-OFF in the forward and reverse orientations in the vector pcDNA-neo (InVitrogen). Cells were subjected to selection in the presence of G418 (Geneticin, GIBCO) for several weeks until individual colonies were visible by the naked eye. These clones were isolated and amplified.

RNA Blot Analyses

Rat tissue polyadenylated mRNA was prepared by the lithium chloride/urea method (Auffrey, C. and F. Raugeon, Eur. J. Biochemistry 107:303-313 (1980), followed by oligo-dT cellulose chromatography (Aviv and Leder, 1972). Polyadenylated mRNA from cell lines was prepared by the proteinase K/SDS method (Gonda, T.J. et al., Molec. Cell. Biol. 2:617-624 (1982)). Samples of mRNA were resolved by electrophoresis on 1% agarose-2.2M formaldehyde gels, blotted onto nylon membranes (Biotrns, ICN) and incubated

with the 2.9 kb insert of clone Re-OF labeled with ^{32}P by random priming as probe (Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, 2d Ed., Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press, (1989). Hybridizations were performed at 42°C in hybridization buffer containing 50% formamide overnight, and blots were washed at 55°C in 0.2X SSC, 0.1% SDS, before exposure to X-AR film at -70°C.

Example 1. Production of Anti-Type III Receptor Protein Antibodies and Microsequencing and Microsequencing of Peptides Resulting from Partial Proteolysis of Purified Type III Receptor

Initially cellular proteoglycans were purified from human placenta and then subjected to enzymatic deglycosylation with heparitinase and chondroitinase. Protein cores in the molecular weight range of 100-130 kilodaltons were further purified by preparative gel electrophoresis; these should include the type III receptor. This partially purified material was used as an immunogen in mice. After screening 850 hybridoma lines developed from immunized mice, three lines were found to produce antibodies that specifically recognized and immunoprecipitated a deglycosylated polypeptide species of 100-120 kD. This species could be radiolabelled by incubation of whole cells with ^{125}I-TGF-β followed by covalent cross-linking. Its size is consistent with that of the protein core previously reported for the type III receptor. (Massague, J., Annu. Rev. Cell. Biol. 6:597-641 (1990))

Monoclonal antibody 94 was used to purify the type III receptor from rat liver by affinity-chromatography. The purified receptor was subjected to partial proteolysis and the resulting peptides were resolved by high pressure liquid chromatography. Several peptides were subjected to microsequencing and yielded the following oligopeptide sequences:

Peptide I: ILLDPDHPPAL (SEQ ID NO. 5)

Peptide II: QAPFPINFMIA (SEQ ID NO. 6)

Peptide III: QPIVPSVQ (SEQ ID NO. 7)

Peptide IV: FYVEQGYGR (SEQ ID NO. 8)

These peptide sequences were used to construct degenerate oligonucleotides that served either as primers in a cloning strategy using the polymerase chain reaction (PCR) or as probes in screening cDNA libraries. While this strategy was not productive, the oligopeptide sequences proved useful in verifying the receptor clones isolated by the second, alternative strategy (see Example 2).

Example 2. Expression Cloning of the Type III Receptor cDNA

An expression cloning strategy in COS cells, a procedure which takes advantage of the considerable amplification of individual cDNAs in transfected COS cells was used as an alternative means to isolate TGF-β receptor clones. Such amplification is mediated by SV40 large T antigen expressed by the COS cells interacting

with a SV40 origin of replication in the cDNA vector. Gearing, D. et al., EMBO J. 8:3667-3676 (1989); Lin, H.Y., et al., Proc. Natl. Acad. Sci. 88:3185-3189 (1991a); Lin, H. Y. et al., Science, in press (1991); Mathews, L. S. and Vale, W. W., Cell 65:973-982 (1991).

The strategy involved the construction of a cDNA library from A-10 cells, a rat vascular smooth muscle cell line that expresses all three high-affinity TGF-β receptors. The resulting cDNAs were inserted into the vector pcDNA-1, which carries the CMV transcriptional promoter and the SV40 origin of replication. The resulting library was then divided into pools of 10,000 independent recombinants each and DNA from each pool was transfected into 1.5 x 10^6 COS-7 cells grown on glass flaskettes by means of DEAE-dextran transfection procedure. Aruffo, A. and Seed, B., Proc. Natl. Acad. Sci., U.S.A. 84:8573-8577 (1987); Gearing, D. et al., EMBO J. 8:3667-3676 (1989); Mathews, L. S. and Vale, W. W., Cell 65:973-982 (1991). The transfected cells were cultured for 48-60 hours and then exposed to radiolabelled TGF-β1 for four hours. Following this treatment, the glass slides carrying these cells were washed extensively and fixed. These slides were dipped in liquid photographic emulsion and examined by darkfield microscopy. While all of the receptor genes cloned to date by this procedure have undetectable or low constitutive levels of expression in COS cells, we were hindered by the fact that untransfected COS cells already express substantial amounts of type III TGF-β receptor. Such expression, estimated to be approximately 2 x 10^5 receptor molecules per cell, might well have generated an unacceptably high level of background binding. However, since the detection procedure involves visualizing radiolabelled

ligand-binding on individual cells, it was hoped that identifying occasional cells expressing substantially higher levels of vector-encoded receptor would be possible. This hope was vindicated in the initial experiments.

After screening nearly one million cDNA clones in this manner, a glass slide containing 20 positive transfectants was identified. The original pool of expression constructs from which one such transfectant was derived was split into 25 subpools of 1000 clones each and these were subjected to a second round of screening. Two further rounds of sib-selection resulted in the isolation of a cDNA clone (R3-OF) with a 2.9 kb insert that induced high levels of TGF-β-binding proteins in approximately 10% of COS cells into which it was transfected.

The specificity of this binding was validated by showing that addition of a 200-fold excess of unlabeled TGF-β competitor strongly reduced binding of ^{125}I-TGF-β to transfected cells. By taking into account a transfection efficiency of 10% and the high background of endogenous receptor expression, we calculated that the level of total ^{125}I-TGF-β binding to each glass slide of cells transfected with this cDNA clone (Figure 1C) was only 2-fold above the level seen with mock transfectants (data not shown). Nonetheless, this marginal increase in ligand-binding was sufficient to identify rare transfectants amidst a large field of cells expressing this background level of receptor.

The R3-OF cDNA encoded an open reading frame of 836 amino acid residues of which the 3′ most 18 were encoded by vector sequence, clearly indicating that clone R3-OF

was an incomplete cDNA insert which ended prematurely at the codon specifying alanine 818 (Figure 4). R3-OF was used as a probe to isolate a full-length cDNA from a rat 208F lambda phage library. This clone, termed R3-OFF, was 6 kb in length and encoded a protein of 853 amino acids; its sequence was co-linear with that of clone R3-OF.

Example 3. Characterization of the Product of the Full Length Clone R3-OFF

Characterization of the product of the full length clone R3-OFF was undertaken in order to determine which of the three TGF-β receptors it specified. To do so, COS transfectants were incubated with radioiodinated TGF-β, chemical crosslinker was added and the labelled receptors were resolved by polyacrylamide gel electrophoresis.

Labelling of cell surface TGF-β receptors in this way resulted in the detection of three distinct species on the surface of COS cells, as extensively by others (Massague, J. et al., Ann. NY Acad. Sci. 593:59-72 (1990). These included the two lower molecular weight type I and II receptors (65 and 85 kD) and the higher molecular weight type III proteoglycan, which migrated as a diffuse band of 280-330 kd. Enzymatic treatment of the proteoglycan with chondroitinase and heparitinase yielded a core protein of approximately 100 kd. Binding to all three receptor types was specific, in that it was completed by 200-fold excess of unlabeled TGF-β1.

Transfecting the R3-OFF cDNA caused a two-fold increase in expression of the type III receptor. When a cell lysate derived from COS cells transfected with clone

R3-OFF was treated with deglycosylating enzymes, the heterogenous 280-330 kd band was converted to a protein core which co-migrated with the type III protein core seen in untransfected A10 cells. Importantly, the recombinant protein core migrates differently from the endogenous COS cell type III protein core.

These observations were confirmed and extended in experiments using stably transfected cells expressing the R3-OFF cDNA. L6 rat skeleton muscle myoblasts normally do not express detectable type III mRNA or endogenous type III receptor (determined by radiolabelled ligand-binding assay). Such cells were transfected with the isolated cDNA in the vector pcDNA-neo. Cell clones stably expressing this clone in both the forward and reverse orientations with respect to the CMV promoter were isolated and analyzed by ligand-binding assay.

Introduction of either the full length clone R3-OFF or the partial clone R3-OF in the forward orientation led to the de novo expression of the type III receptor. L6 cells transfected with the cDNA in reversed orientation did not express this protein. The apparent size of the protein core of the type III receptor in cells transfected with the R3-OF clone is smaller than that expressed by R3-OFF transfected cells, consistent with the difference in the sizes of the protein cores predicted from the respective nucleic acid sequences (Figure 1).

Unexpectedly, the amount of radio-labelled ligand corss-linked to the type II receptor is increased by 2.5 fold in cells expressing the type III cDNA, while the amount cross-linked to the type I receptor remained

unchanged. This apparent specific up-regulation of ligand-binding to the type II receptor could be detected with all of the 15 stably transfected L6 cell lines analyzed so far. This effect seems to be mediated by the truncated clone R3-OF which lacks the cytoplasmic domain as well as by the full-length clone R3-OFF.

Example 4. Expression of Type III Receptor

Northern blot analysis demonstrated that the type III receptor mRNA is expressed as a single 6 kb message in several rat tissues. The level of mRNA expression in the liver was less than in other tissues, a result expected from earlier surveys of various tissues using radioiodinated TGF-β1. Based on this information, it appears that clone R3-OFF, with a ~6 kb cDNA insert, represents a full length rat type III cDNA clone.

Cells of mouse origin (MEL and YH16) appear to express a smaller (~5.5 kb) message for the type III mRNA than those of pig, rat and human origin. In all of these cells, expression or absence of the type III mRNA is consistent with the expression or absence of detectable cell surface type III receptors with the notable exception of the retinoblastoma cell lines (Y79, Weri-1, Weri-24, and Weri-27). These cells have previously been shown to lack detectable surface expression of type III receptor, a result confirmed by our own unpublished work. It is striking that the type III receptor mRNA is expressed in these cells at a level comparable to that of other cells that do indeed express type III receptor proteins at readily detectable levels. At this moment, we can only conclude that TGF-β receptor III expression,

which is substantial in normal retinoblasts (AD12), has been down-regulated in these retinoblastoma tumor cells, perhaps through post-transcriptional mechanisms.

Example 5. Sequence Analysis of the Full-Length Type III cDNA

The full-length cDNA clone (R3-OFF), described in Example 4, was subjected to sequence analysis. The full reading frame along with flanking sequences is presented in Figure 1. This reading frame encodes a protein of 853 amino acid residues, which is compatible with the 100 kD observed for the fully deglycosylated TGF-β type III receptor.

Two segments of derived protein sequence (underlined and italicized, residues 378-388 and 427-434) precisely match those determined earlier from direct biochemical analysis of the purified receptor protein. This further secured the identity of this isolated cDNA clone as encoding the rat type III receptor.

This TGF-β binding protein has an unusual structure for a cytokine receptor. Hydropathy analysis indicates a N-terminal signal sequence, followed by a long, hydrophilic N-terminal region (Kyte, J. and R. F. Doolittle, J. Mol. Biol. 157:105-132 (1982)). A 27 residue region of strong hydrophobicity (underlined, residues 786-812) toward the C-terminus represents the single putative transmembrane domain. This suggests that nearly all of the receptor is composed of an N-terminal extracellular domain that is anchored to the plasma membrane near its C-terminus. A relatively short

C-terminal tail of 41 residues represents the putative cytoplasmic domain.

The clone R3-OF was also analyzed and found to be a truncated version of R3-OFF, with an identical open reading frame but whose last encoded residue is alanine 818 (Figure 1).

In R3-OFF there are six consensus N-linked glycosylation sites and 15 cysteines (indicated in Figure 1). There is at least one consensus glycosaminoglycan addition site at serine 535 (Bernfield, M. and K. C. Hooper, Ann. N.Y. Acad. Sci. in press (1991), and numerous Ser-Gly residues that are potential sites for GAG conjugation. A consensus protein kinase C site is also present at residue 817.

Only one other gene described to date, a glycoprotein expressed in high quantities by endothelial cells and termed endoglin (Gougos and Letarte, 1990), contains a related amino acid sequence. Overall, there is ~30% identity with the type III receptor over the entire 645 amino acid residue length of endoglin. The most homologous regions between the sequences of the type III receptor and endoglin (74% identity) falls primarily in the putative transmembrane and cytoplasmic domains. Similar to the general structure of type III receptor, endoglin is a glycoprotein which contains a large hydrophilic and presumably extracellular N-terminal domain followed by a putative transmembrane domain and a short cytoplasmic tail of 47 amino acid residues. The biological role of endoglin is unclear, though it has been suggested that it may involve cell-cell recognition through interactions of an "RGD" sequence on its

ectodomain with other adhesion molecules. Unlike the TGF-β type III receptor, endoglin does not carry GAG groups.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using not more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

International Application No: PCT/

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 6, line 12 of the description [1]

A. IDENTIFICATION OF DEPOSIT [3]

Further deposits are identified on an additional sheet ☐ [3]

Name of depositary institution [4]

American Type Culture Collection

Address of depositary institution (including postal code and country) [4]

12301 Parklawn Drive
Rockville, Maryland 20852 USA

Date of deposit [5]	Accession Number [6]
21 October 1991	75127

B. ADDITIONAL INDICATIONS [7] (leave blank if not applicable). This information is continued on a separate attached sheet ☐

In respect of those designations in which a European Patent is sought, the Applicant hereby informs the European Patent Office under European Rule 28(4) that, until the publication of the mention of the grant of the European Patent or until the date on which the European Application has been refused or is withdrawn or is deemed to be withdrawn, the availability of the biological material deposited with the American Type Culture Collection under Accession No. shall be effected only by the issue of a sample to an expert nominated by the requester in accordance with European Rule 28(5).

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE [8] (if the indications are not for all designated States)

Europe (EP)
Australia
Canada
Japan

D. SEPARATE FURNISHING OF INDICATIONS [9] (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later [9] (Specify the general nature of the indications e.g., "Accession Number of Deposit")

E. ☑ This sheet was received with the international application when filed (to be checked by the receiving Office)

YVONNE L. BONNER
INTERNATIONAL DIVISION
(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau [10]

was

(Authorized Officer)

CLAIMS

1. Isolated DNA encoding TGF-β receptor of vertebrate origin or DNA which hybridizes thereto and encodes TGF-β receptor of vertebrate origin.

2. Isolated DNA of Claim 1 wherein the TGF-β receptor is TGF-β type III receptor or TGF-β type II receptor.

3. Isolated DNA of Claim 2 which is of mammalian origin.

4. Isolated DNA of murine or human origin encoding TGF-β type III receptor or DNA which hybridizes thereto.

5. Isolated DNA of Claim 4 having the nucleotide sequence of Figure 1 or a portion thereof sufficient to encode TGF-β type III receptor.

6. Isolated DNA of murine or human origin encoding TGF-β type II receptor or DNA which hybridizes thereto.

7. Isolated DNA of Claim 6 having the nucleotide sequence of Figure 2 or a portion thereof sufficient to encode TGF-β type II receptor.

8. Isolated TGF-β type III receptor of mammalian origin.

9. Isolated TGF-β type III receptor of Claim 8 having the amino acid sequence of Figure 1 or a substantially similar amino acid sequence.

10. Isolated TGF-β type II receptor of mammalian origin.

11. Isolated TGF-β type II receptor of Claim 10 having the amino acid sequence of Figure 3 or a substantially similar amino acid sequence.

12. Recombinant TGF-β type III receptor of mammalian origin.

13. Recombinant TGF-β type III receptor of Claim 8 having the amino acid sequence of Figure 1 or a substantially similar amino acid sequence.

14. Recombinant TGF-β type II receptor of mammalian origin.

15. Recombinant TGF-β type II receptor of Claim 10 having the amino acid sequence of Figure 4 or a substantially similar amino acid sequence.

16. Soluble TGF-β receptor.

17. Soluble TGF-β receptor of Claim 16 which is soluble TGF-β type III receptor.

18. Soluble TGF-β type III receptor of Claim 17 in which the amino acid sequence is amino acids 1 through

785, inclusive, of Figure 1 or a substantially similar amino acid sequence.

19. Soluble TGF-β receptor of Claim 16 which is soluble TGF-β type II receptor.

20. Soluble TGF-β receptor of Claim 19 in which the amino acid sequence is approximately amino acids 1 through 166, inclusive, of Figure 3, or a substantially similar amino acid sequence.

21. An antibody which specifically recognized TGF-β type III receptor of mammalian origin.

22. An antibody of Claim 21 which is a monoclonal antibody.

23. An antibody which specifically recognizes soluble TGF-β type III receptor of mammalian origin.

24. An antibody which specifically recognizes soluble TGF-β type II receptor of mammalian origin.

25. A method of altering TGF-β binding to TGF-β type II or type III receptor on the surface of a cell, comprising combining soluble TGF-β type II or type III receptor with the cell, under conditions appropriate for binding of the soluble TGF-β receptor and TGF-β.

26. The method of Claim 25 wherein TGF-β binding is inhibited.

27. A method of altering TGF-β binding to TGF-β type III receptor on the surface of a cell comprising combining the cell with DNA encoding TGF-β type III receptor in an appropriate expression system which expresses TGF-β type III receptor, under conditions appropriate for expression of TGF-β type III receptor and binding of TGF-β with TGF-β type III receptor.

28. A method of regulating the effect of TGF-β in a mammal, comprising administering to the mammal a TGF-β receptor selected from the group consisting of: TGF-β type III receptor, TGF-β type II receptor, soluble TGF-β type III receptor, soluble TGF-β type II receptor, TGF-β bound to TGF-β type III receptor or a combination thereof, in sufficient quantity to alter binding of TGF-β to TGF-β type III receptor, binding of TGF-β to TGF-β type II receptor or both.

29. TGF-β receptor according to any one of Claims 8 to 20, for use in therapy.

30. An antibody according to any one of Claims 21 to 24, for use in therapy.

31. Use of TGF-β receptor according to any one of Claims 8 to 20, for the manufacture of a medicament for altering (e.g. inhibiting) TGF-β binding to TGF-β type II or type III receptor on the surface of a cell.

32. Use of a TGF-β receptor selected from the group consisting of: TGF-β type III receptor, TGF-β type II receptor, soluble TGF-β type III receptor, soluble TGF-β type II receptor, TGF-β bound to TGF-β type III receptor or a combination thereof, for the manufacturing of a medicament for use in regulating the affect of TGF-β in a mammal.

33. A method of assessing the ability of a compound to interfere with TGF-β binding to the TGF-β type III receptor, comprising the steps of:

a) combining:

1) mammalian cells which express the TGF-β type III receptor;

2) labeled TGF-β; and

3) a compound to be assessed;

b) maintaining the product of (a) under conditions sufficient for TGF-β to bind to the TGF-β type III receptor;

c) determining the extent of binding of TGF-β to TGF-β type III receptors in the presence of the compound to be assessed; and

d) comparing the determination made in (c) with the extent to which binding of TGF-β to the TGF-β type III receptor occurs in the absence of the compound to be assessed,

wherein if TGF-β binding to the TGF-β type III receptor occurs to a lesser extent in the presence of the compound to be assessed than in the absence of the compound to be assessed, the compound to be assessed interferes with TGF-β binding to TGF-β type III receptors.

34. A method of Claim 33 wherein the cells which express the TGF-β type III receptor are a cell line.

35. A method of Claim 34 wherein the cells which express the TGF-β type III receptor are cells modified to express the TGF-β type III receptor.

36. A method of Claim 35 wherein the cells modified to express the TGF-β type III receptor are cells which have incorporated into them TGF-β receptor cDNA in an appropriate vector or microinjected TGF-β receptor RNA.

37. A method of assessing the ability of a compound to interfere with TGF-β binding to the TGF-β type II receptor comprising the steps of:

a) combining:

   1) mammalian cells which express the TGF-β type II receptor;

   2) labeled TGF-β; and

   3) a compound to be assessed;

b) maintaining the product of (a) under conditions sufficient for TGF-β to bind to the TGF-β type II receptor;

c) determining the extent of binding of TGF-β to TGF-β type II receptors in the presence of the compound to be assessed; and

d) comparing the determination made in (c) with the extent to which binding of TGF-β to the TGF-β type II receptor occurs in the absence of the compound to be assessed,

wherein if TGF-β binding to the TGF-β type II receptor occurs to a lesser extent in the presence of the compound to be assessed than in the absence of the compound to be assessed, the compound to be assessed has interfered with TGF-β binding to TGF-β type II receptor.

38. A method of Claim 37 wherein the cells which express the TGF-β type II receptor are a cell line.

39. A method of Claim 38 wherein the cells which express the TGF-β type II receptor are cells modified to express the TGF-β type II receptor.

40. A method of Claim 39 wherein the cells modified to express the TGF-β type II receptor are cells which have incorporated into them TGF-β receptor cDNA in an appropriate vector or microinjected TGF-β receptor RNA.

41. A method of detecting abnormal binding of TGF-β TGF-β type III receptors of TGF-β type II receptors at a cell surface, comprising:

a) determining the extent of binding of TGF-β to TGF-β type III receptors or TGF-β type II receptors by cells in a sample obtained from an individual in whom binding is to be assessed thereby producing a test binding value; and

b) comparing the results of (a) with the extent to which binding occurs at the cell surface in control cells which are cells known to have abnormal binding of TGF-β to TGF-β type III receptors or TGF-β type II receptors resulting in a control binding value,

wherein a test binding value similar to the control binding value is indicative of abnormal binding of TGF-β to TGF-β type III receptor or TGF-β type II receptor.

FIGURE 1A

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-240
CAGGAGGTGA AAGTCCCCGG CGGTCCGGAT GGCGCAGTTG CACTGCGCTG CTGAGCTCGC -180
GGCCGCCTGC GCACACTGGG GGGACTCGCT TCGGCTAGTA ACTCCTCCAC CTCGCGGCGG -120
ACGACCGGTC CTGGACACGC TGCCTGCGAG GCAAGTTGAA CAGTGCAGAG AAGGATCTTA - 60
AAGCTACACC CGACTTGCCA CGATTGCCTT CAATCTGAAG AACCAAAGGC TGTTGGAGAG -  1
                                             ---

ATG GCA GTG ACA TCC CAC CAC ATG ATC CCG GTG ATG GTT GTC CTG ATG      48
Met Ala Val Thr Ser His His Met Ile Pro Val Met Val Val Leu Met      16

AGC GCC TGC CTG GCC ACC GCC GGT CCA GAG CCC AGC ACC CGG TGT GAA      96
Ser Ala Cys Leu Ala Thr Ala Gly Pro Glu Pro Ser Thr Arg Cys Glu      32
                            †                           &

CTG TCA CCA ATC AAC GCC TCT CAC CCA GTC CAG GCC TTG ATG GAG AGC     144
Leu Ser Pro Ile Asn Ala Ser His Pro Val Gln Ala Leu Met Glu Ser      48
                    #

TTC ACC GTT CTG TCT GGC TGT GCC AGC AGA GGC ACC ACC GGG CTG CCA     192
Phe Thr Val Leu Ser Gly Cys Ala Ser Arg Gly Thr Thr Gly Leu Pro      64
                    +       &

AGG GAG GTC CAT GTC CTA AAC CTC CGA AGT ACA GAT CAG GGA CCA GGC     240
Arg Glu Val His Val Leu Asn Leu Arg Ser Thr Asp Gln Gly Pro Gly      80

CAG CGG CAG AGA GAG GTT ACC CTG CAC CTG AAC CCC ATT GCC TCG GTG     288
Gln Arg Gln Arg Glu Val Thr Leu His Leu Asn Pro Ile Ala Ser Val      96

CAC ACT CAC CAC AAA CCT ATC GTG TTC CTG CTC AAC TCC CCC CAG CCC     336
His Thr His His Lys Pro Ile Val Phe Leu Leu Asn Ser Pro Gln Pro     112

CTG GTG TGG CAT CTG AAG ACG GAG AGA CTG GCC GCT GGT GTC CCC AGA     384
Leu Val Trp His Leu Lys Thr Glu Arg Leu Ala Ala Gly Val Pro Arg     128

CTC TTC CTG GTT TCG GAG GGT TCT GTG GTC CAG TTT CCA TCA GGA AAC     432
Leu Phe Leu Val Ser Glu Gly Ser Val Val Gln Phe Pro Ser Gly Asn     144
                                                    +       #

TTC TCC TTG ACA GCA GAA ACA GAG GAA AGG AAT TTC CCT CAA GAA AAT     480
Phe Ser Leu Thr Ala Glu Thr Glu Glu Arg Asn Phe Pro Gln Glu Asn     160

GAA CAT CTC GTG CGC TGG GCC CAA AAG GAA TAT GGA GCA GTG ACT TCG     528
Glu His Leu Val Arg Trp Ala Gln Lys Glu Tyr Gly Ala Val Thr Ser     176

TTC ACT GAA CTC AAG ATA GCA AGA AAC ATC TAT ATT AAA GTG GGA GAA     576
Phe Thr Glu Leu Lys Ile Ala Arg Asn Ile Tyr Ile Lys Val Gly Glu     192
```

FIGURE 1B

GAT CAA GTG TTT CCT CCT ACG TGT AAC ATA GGG AAG AAT TTC CTC TCA 624
Asp Gln Val Phe Pro Pro Thr Cys Asn Ile Gly Lys Asn Phe Leu Ser 208
&

CTC AAT TAC CTT GCC GAG TAC CTT CAA CCC AAA GCC GCC GAA GGT TGT 672
Leu Asn Tyr Leu Ala Glu Tyr Leu Gln Pro Lys Ala Ala Glu Gly Cys 224
&

GTC CTG CCC AGT CAG CCC CAT GAA AAG GAA GTA CAC ATC ATC GAG TTA 720
Val Leu Pro Ser Gln Pro His Glu Lys Glu Val His Ile Ile Glu Leu 240

ATT ACC CCC AGC TCG AAC CCT TAC AGC GCT TTC CAG GTG GAT ATA ATA 768
Ile Thr Pro Ser Ser Asn Pro Tyr Ser Ala Phe Gln Val Asp Ile Ile 256

GTT GAC ATA CGA CCT GCT CAA GAG GAT CCC GAG GTG GTC AAA AAC CTT 816
Val Asp Ile Arg Pro Ala Gln Glu Asp Pro Glu Val Val Lys Asn Leu 272

GTC CTG ATC TTG AAG TGC AAA AAG TCT GTC AAC TGG GTG ATC AAG TCT 864
Val Leu Ile Leu Lys Cys Lys Lys Ser Val Asn Trp Val Ile Lys Ser 288
&

TTT GAC GTC AAG GGA AAC TTG AAA GTC ATT GCT CCC AAC AGT ATC GGC 912
Phe Asp Val Lys Gly Asn Leu Lys Val Ile Ala Pro Asn Ser Ile Gly 304

TTT GGA AAA GAG AGT GAA CGA TCC ATG ACA ATG ACC AAA TTG GTA AGA 960
Phe Gly Lys Glu Ser Glu Arg Ser Met Thr Met Thr Lys Leu Val Arg 320

GAT GAC ATC CCT TCC ACC CAA GAG AAT CTG ATG AAG TGG GCA CTG GAC 1008
Asp Asp Ile Pro Ser Thr Gln Glu Asn Leu Met Lys Trp Ala Leu Asp 336

AAT GGC TAC AGG CCA GTG ACG TCA TAC ACA ATG GCT CCC GTG GCT AAT 1056
Asn Gly Tyr Arg Pro Val Thr Ser Tyr Thr Met Ala Pro Val Ala Asn 352

AGA TTT CAT CTT CGG CTT GAG AAC AAC GAG GAG ATG AGA GAT GAG GAA 1104
Arg Phe His Leu Arg Leu Glu Asn Asn Glu Glu Met Arg Asp Glu Glu 368

GTC CAC ACC ATT CCT CCT GAG CTT CGT ATC CTG CTG GAC CCT GAC CAC 1152
Val His Thr Ile Pro Pro Glu Leu Arg Ile Leu Leu Asp Pro Asp His 384
peptide 1

CCG CCC GCC CTG GAC AAC CCA CTC TTC CCA GGA GAG GGA AGC CCA AAT 1200
Pro Pro Ala Leu Asp Asn Pro Leu Phe Pro Gly Glu Gly Ser Pro Asn 400

GGT GGT CTC CCC TTT CCA TTC CCG GAT ATC CCC AGG AGA GGC TGG AAG 1248
Gly Gly Leu Pro Phe Pro Phe Pro Asp Ile Pro Arg Arg Gly Trp Lys 416

GAG GGC GAA GAT AGG ATC CCC CGG CCA AAG CAG CCC ATC GTT CCC AGT 1296
Glu Gly Glu Asp Arg Ile Pro Arg Pro Lys Gln Pro Ile Val Pro Ser 432
peptide 2

FIGURE 1C

```
GTT CAA CTG CTT CCT GAC CAC CGA GAA CCA GAA GAA GTG CAA GGG GGC   1344
Val Gln Leu Leu Pro Asp His Arg Glu Pro Glu Glu Val Gln Gly Gly    448

GTG GAC ATC GCC CTG TCA GTC AAA TGT GAC CAT GAA AAG ATG GTC GTG   1392
Val Asp Ile Ala Leu Ser Val Lys Cys Asp His Glu Lys Met Val Val    464
                                 &

GCT GTA GAC AAA GAC TCT TTC CAG ACC AAT GGC TAC TCA GGG ATG GAG   1440
Ala Val Asp Lys Asp Ser Phe Gln Thr Asn Gly Tyr Ser Gly Met Glu    480
                                                 +

CTC ACC CTG TTG GAT CCT TCG TGT AAA GCC AAA ATG AAT GGT ACT CAC   1488
Leu Thr Leu Leu Asp Pro Ser Cys Lys Ala Lys Met Asn Gly Thr His    496
                             &                   #

TTT GTT CTC GAG TCT CCC CTG AAT GGC TGT GGT ACT CGA CAT CGG AGG   1536
Phe Val Leu Glu Ser Pro Leu Asn Gly Cys Gly Thr Arg His Arg Arg    512
                                     &

TCG ACC CCG GAT GGT GTG GTT TAC TAT AAC TCT ATT GTG GTG CAG GCT   1584
Ser Thr Pro Asp Gly Val Val Tyr Tyr Asn Ser Ile Val Val Gln Ala    528

CCG TCC CCT GGG GAT AGC AGT GGC TGG CCT GAT GGC TAT GAA GAC TTG   1632
Pro Ser Pro Gly Asp Ser Ser Gly Trp Pro Asp Gly Tyr Glu Asp Leu    544
                        +++

GAG TCA GGC GAT AAT GGA TTT CCT GGA GAC GGG GAT GAA GGA GAA ACT   1680
Glu Ser Gly Asp Asn Gly Phe Pro Gly Asp Gly Asp Glu Gly Glu Thr    560
     +

GCC CCC CTG AGC CGA GCT GGA GTG GTG GTG TTT AAC TGC AGC TTG CGG   1728
Ala Pro Leu Ser Arg Ala Gly Val Val Val Phe Asn Cys Ser Leu Arg    576
                                             #   &

CAG CTG AGG AAT CCC AGT GGC TTC CAG GGC CAG CTC GAT GGA AAT GCT   1776
Gln Leu Arg Asn Pro Ser Gly Phe Gln Gly Gln Leu Asp Gly Asn Ala    592
                                                         #

ACC TTC AAC ATG GAG CTG TAT AAC ACA GAC CTC TTT CTG GTG CCC TCC   1824
Thr Phe Asn Met Glu Leu Tyr Asn Thr Asp Leu Phe Leu Val Pro Ser    608

CCA GGG GTC TTC TCT GTG GCA GAG AAC GAG CAT GTT TAT GTT GAG GTG   1872
Pro Gly Val Phe Ser Val Ala Glu Asn Glu His Val Tyr Val Glu Val    624

TCT GTC ACC AAG GCT GAC CAA GAT CTG GGA TTC GCC ATC CAA ACC TGC   1920
Ser Val Thr Lys Ala Asp Gln Asp Leu Gly Phe Ala Ile Gln Thr Cys    640
                                                             &
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FIGURE 1D

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TTT CTC TCT CCA TAC TCC AAC CCA GAC AGA ATG TCT GAT TAC ACC ATC   1968
Phe Leu Ser Pro Tyr Ser Asn Pro Asp Arg Met Ser Asp Tyr Thr Ile    656

ATC GAG AAC ATC TGT CCG AAA GAC GAC TCT GTG AAG TTC TAC AGC TCC   2016
Ile Glu Asn Ile Cys Pro Lys Asp Asp Ser Val Lys Phe Tyr Ser Ser    672
                 &

AAG AGA GTG CAC TTT CCC ATC CCG CAT GCT GAG GTG GAC AAG AAG CGC   2064
Lys Arg Val His Phe Pro Ile Pro His Ala Glu Val Asp Lys Lys Arg    688

TTC AGC TTC CTG TTC AAG TCT GTG TTC AAC ACC TCC CTG CTC TTC CTG   2112
Phe Ser Phe Leu Phe Lys Ser Val Phe Asn Thr Ser Leu Leu Phe Leu    704
                                     #

CAC TGC GAG TTG ACT CTG TGC TCC AGG AAG AAG GGC TCC CTG AAG CTG   2160
His Cys Glu Leu Thr Leu Cys Ser Arg Lys Lys Gly Ser Leu Lys Leu    720
     &                   &

CCG AGG TGT GTG ACT CCT GAC GAC GCC TGC ACT TCT CTC GAT GCC ACC   2208
Pro Arg Cys Val Thr Pro Asp Asp Ala Cys Thr Ser Leu Asp Ala Thr    736
         &                           &

ATG ATC TGG ACC ATG ATG CAG AAT AAG AAG ACA TTC ACC AAG CCC CTG   2256
Met Ile Trp Thr Met Met Gln Asn Lys Lys Thr Phe Thr Lys Pro Leu    752

GCT GTG GTC CTC CAG GTA GAC TAT AAA GAA AAT GTT CCC AGC ACT AAG   2304
Ala Val Val Leu Gln Val Asp Tyr Lys Glu Asn Val Pro Ser Thr Lys    768

GAT TCC AGT CCA ATT CCT CCT CCT CCT CCA CAG ATT TTC CAT GGC CTG   2352
Asp Ser Ser Pro Ile Pro Pro Pro Pro Pro Gln Ile Phe His Gly Leu    784

GAC ACG CTC ACC GTG ATG GGC ATT GCA TTT GCA GCA TTT GTG ATC GGA   2400
Asp Thr Leu Thr Val Met Gly Ile Ala Phe Ala Ala Phe Val Ile Gly    800

GCG CTC CTG ACG GGG GCC TTG TGG TAC ATC TAC TCC CAC ACA GGG GAG   2448
Ala Leu Leu Thr Gly Ala Leu Trp Tyr Ile Tyr Ser His Thr Gly Glu    816

ACA GCA CGA AGG CAG CAA GTC CCT ACC TCG CCG CCA GCC TCG GAG AAC   2496
Thr Ala Arg Arg Gln Gln Val Pro Thr Ser Pro Pro Ala Ser Glu Asn    832
 $   @

AGC AGC GCG GCC CAC AGC ATC GGC AGC ACT CAG AGT ACC CCC TGC TCT   2544
Ser Ser Ala Ala His Ser Ile Gly Ser Thr Gln Ser Thr Pro Cys Ser    848

AGC AGC AGC ACA GCC TAGGTGGACA GACAGACGCC CGCCCACCGC AGCCAGGGCA   2599
Ser Ser Ser Thr Ala***                                             853
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FIGURE 1E

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GGGCCCGATG CCAGTGCTGC GTGTCCACAG TCAGAAGTCT TGATCTGGGC TCCCTGTAAA 2659
GAAAGAGTGA ATTTCAGTAT ACAGACAGCC AGTTCTACCC ACCCCTTACC ACGGCCCACA 2719
TAAATGTGAC CCTGGGCATC TGTCACACGA AAGCTAAGCT GGTGGCCTTC CCCACCAGCC 2779
CCTCGCAGGA TGGGGGTTTC AATGTGAAAC ATCTGCCAGT TTTGTTTTGT TTTTTTAATG 2839
CTGCTTTGTC CAGGTGTCCA AACATCCATC ATTTGGGGTG GTCTGTTTTA CAGAGTAAAG 2899
GAGGCGGTGA AGGGACGTCA GCTAGTGTGT AGAGCCAAGG GGAGACAGCT AGGATTCTCG 2959
CCTAGCTGAA CCAAGGTGTA AAATAGAAGA CACGCTCC                         2997
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FIGURE 2

				GTTGGCGAGG
AGTTTCCTGT	TTCCCCCGCA	GCGCTGAGTT	GAAGTTGAGT	GAGTCACTCG
CGCGCACGGA	GCGACGACAC	CCCCGCGCGT	GCACCCGCTC	GGGACAGGAG
CCGGACTCCT	GTGCAGCTTC	CCTCGGCCGC	CGGGGGCCTC	CCCGCGCCTC
GCCGGCCTCC	AGGCCCCTCC	TGGCTGGCGA	GCGGGCGCCA	CATCTGGCCC
GCACATCTGC	GCTGCCGGCC	CGGCGCGGGG	TCCGGAGAGG	GCGCGGCGCG
GAGCGCAGCC	AGGGGTCCGG	GAAGGCGCCG	TCCGTGCGCT	GGGGGCTCGG
TCTATGACGA	GCAGCGGGGT	CTGCCATGGG	TCGGGGGCTG	CTCAGGGGCC
TGTGGCCGCT	GCACATCGTC	CTGTGGACGC	GTATCGCCAG	CACGATCCCA
CCGCACGTTC	AGAAGTCGGT	TAATAACGAC	ATGATAGTCA	CTGACAACAA
CGGTGCAGTC	AAGTTTCCAC	AACTGTGTAA	ATTTTGTGAT	GTGAGATTTT
CCACCTGTGA	CAACCAGAAA	TCCTGCATGA	GCAACTGCAG	CATCACCTCC
ATCTGTGAGA	AGCCACAGGA	AGTCTGTGTG	GCTGTATGGA	GAAAGAATGA
CGAGAACATA	ACACTAGAGA	CAGTTTGCCA	TGACCCCAAG	CTCCCCTACC
ATGACTTTAT	TCTGGAAGAT	GCTGCTTCTC	CAAAGTGCAT	TATGAAGGAA
AAAAAAAAGC	CTGGTGAGAC	TTTCTTCATG	TGTTCCTGTA	GCTCTGATGA
GTGCAATGAC	AACATCATCT	TCTCAGAAGA	ATATAACACC	AGCAATCCTG
ACTTGTTGCT	AGTCATATTT	CAAGTGACAG	GCATCAGCCT	CCTGCCACCA
CTGGGAGTTG	CCATATCTGT	CATCATCATC	TTCTACTGCT	ACCGCGTTAA
CCGGCAGCAG	AAGCTGAGTT	CAACCTGGGA	AACCGGCAAG	ACGCGGAAGC
TCATGGAGTT	CAGCGAGCAC	TGTGCCATCA	TCCTGGAAGA	TGACCGCTCT
GACATCAGCT	CCACGTGTGC	CAACAACATC	AACCACAACA	CAGAGCTGCT
GCCCATTGAG	CTGGACACCC	TGGTGGGGAA	AGGTCGCTTT	GCTGAGGTCT
ATAAGGCCAA	GCTGAAGCAG	AACACTTCAG	AGCAGTTTGA	GACAGTGGCA
GTCAAGATCT	TTCCCTATGA	GGAGTATGCC	TCTTGGAAGA	CAGAGAAGGA
CATCTTCTCA	GACATCAATC	TGAAGCATGA	GAACATACTC	CAGTTCCTGA
CGGCTGAGGA	GCGGAAGACG	GAGTTGGGGA	AACAATACTG	GCTGATCACC
GCCTTCCACG	CCAAGGGCAA	CCTACAGGAG	TACCTGACGC	GGCATGTCAT
CAGCTGGGAG	GACCTGCGCA	AGCTGGGCAG	CTCCCTCGCC	CGGGGGATTG
CTCACCTCCA	CAGTGATCAC	ACTCCATGTG	GGAGGCCCAA	GATGCCCATC
GTGCACAGGG	ACCTCAAGAG	CTCCAATATC	CTCGTGAAGA	ACGACCTAAC
CTGCTGCCTG	TGTGACTTTG	GGCTTTCCCT	GCGTCTGGAC	CCTACTCTGT
CTGTGGATGA	CCTGGCTAAC	AGTGGGCAGG	TGGGAACTGC	AAGATACATG
GCTCCAGAAG	TCCTAGAATC	CAGGATGAAT	TTGGAGAATG	CTGAGTCCTT
CAAGCAGACC	GATGTCTACT	CCATGGCTCT	GGTGCTCTGG	GAAATGACAT
CTCGCTGTAA	TGCAGTGGGA	GAAGTAAAAG	ATTATGAGCC	TCCATTTGGT
TCCAAGGTGC	GGGAGCACCC	CTGTGTCGAA	AGCATGAAGG	ACAACGTGTT
GAGAGATCGA	GGGCGACCAG	AAATTCCCAG	CTTCTGGCTC	AACCACCAGG
GCATCCAGAT	GGTGTGTGAG	ACGTTGACTG	AGTGCTGGGA	CCACGACCCA
GAGGCCCGTC	TCACAGCCCA	GTGTGTGGCA	GAACGCTTCA	GTGAGCTGGA
GCATCTGGAC	AGGCTCTCGG	GGAGGAGCTG	CTCGGAGGAG	AAGATTCCTG
AAGACGGCTC	CCTAAACACT	ACCAAATAGC	TCTTATGGGG	CAGGCTGGGC
ATGTCCAAAG	AGGCTGCCCC	TCTCACCAAA		

FIGURE 3

MGRGLLRGLW	PLHIVLWTRI	ASTIPPHVQK	SVNNDMIVTD	NNGAVKFPQL
CKFCDVRFST	CDNQKSCMSN	CSITSICEKP	QEVCVAVWRK	NDENITLETV
CHDPKLPYHD	FILEDAASPK	CIMKEKKKPG	ETFFMCSCSS	DECNDNIIFS
EEYNTSNPDL	LLVIFQVTGI	SLLPPLGVAI	SVIIIFYCYR	VNRQQKLSST
WETGKTRKLM	EFSEHCAIIL	EDDRSDISST	CANNINHNTE	LLPIELDTLV
GKGRFAEVYK	AKLKQNTSEQ	FETVAVKIFP	YEEYASWKTE	KDIFSDINLK
HENILQFLTA	EERKTELGKQ	YWLITAFHAK	GNLQEYLTRH	VISWEDLRKL
GSSLARGIAH	LHSDHTPCGR	PKMPIVHRDL	KSSNILVKND	LTCCLCDFGL
SLRLDPTLSV	DDLANSGQVG	TARYMAPEVL	ESRMNLENAE	SFKQTDVYSM
ALVLWEMTSR	CNAVGEVKDY	EPPFGSKVRE	HPCVESMKDN	VLRDRGRPEI
PSFWLNHQGI	QMVCETLTEC	WDHDPEARLT	AQCVAERFSE	LEHLDRLSGR
SCSEEKIPED	GSLNTTK			

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/12; C12N5/10; C12P21/08; C07K13/00
A61K37/02

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12N ; C07K ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	EP,A,0 369 861 (ONCOGEN LTD PARTNERSHIP, US) 23 May 1990 see the whole document ---	1-41
X	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS. vol. 179, no. 1, 30 August 1991, DULUTH, MINNESOTA US pages 378 - 385 O'GRADY P;HUANG SS;HUANG JS; 'Expression of a new type high molecular weight receptor (type V receptor) of transforming growth factor beta in normal and transformed cells.' see the whole document ---	1,16,29

-/--

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
20 JANUARY 1993	12.02.93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	S.A. NAUCHE

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,X	CELL. vol. 68, no. 4, 21 February 1992, CAMBRIDGE, NA US pages 775 - 785 LIN HY;WANG XF;NG-EATON E;WEINBERG RA;LODISH HF; 'Expression cloning of the TGF-beta type II receptor, a functional transmembrane serine/threonine kinase.' see the whole document	1-3,6,7, 10,11, 14-16, 19,20, 24-26, 28-31 37-41
P,X	CELL. vol. 67, no. 4, 15 November 1991, CAMBRIDGE, NA US pages 785 - 795 LOPEZ-CASILLAS F;CHEIFETZ S;DOODY J;ANDRES JL;LANE WS;MASSAGUE J; 'Structure and expression of the membrane proteoglycan betaglycan, a component of the TGF-beta receptor system.' see the whole document	1-5,8,9, 12,13, 16-18, 21-23, 25-36,41
P,X	CELL. vol. 67, no. 4, 15 November 1991, CAMBRIDGE, NA US pages 797 - 805 WANG XF;LIN HY;NG-EATON E;DOWNWARD J;LODISH HF;WEINBERG RA; 'Expression cloning and characterization of the TGF-beta type III receptor.' see the whole document	1-5,8,9, 12,13, 16-18, 21-23, 25-36,41
A	JOURNAL OF BIOLOGICAL CHEMISTRY. (MICROFILMS) vol. 265, no. 33, 25 November 1990, BALTIMORE, MD US pages 20533 - 20538 CHEIFETZ S;HERNANDEZ H;LAIHO M;TEN DIJKE P;IWATA KK;MASSAGUE J; 'Distinct transforming growth factor-beta (TGF-beta) receptor subsets as determinants of cellular responsiveness to three TGF-beta isoforms.'	
A	JOURNAL OF BIOLOGICAL CHEMISTRY. (MICROFILMS) vol. 263, no. 32, 15 November 1988, BALTIMORE, MD US pages 16984 - 16991 CHEIFETZ S.; ANDRES J.L.; MASSAGUE J; 'The transforming growth factor-beta receptor type III is a membrane proteoglycan. Domain structure of the receptor.'	

-/--

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	ANNALS OF THE NEW YORK ACADEMY OF SCIENCES vol. 593, 1990, NEW YORK, US; pages 59 - 72 MASSAGUÉ, J. ET AL; 'TGF-Beta recepotrs and TGF-beta binding proteoglycans : recent progress in identifying their functional properties.' -----	

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9209326
SA 66669

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 20/01/93
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0369861	23-05-90	CA-A- 2002011	14-05-90

EPO FORM P0479

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82